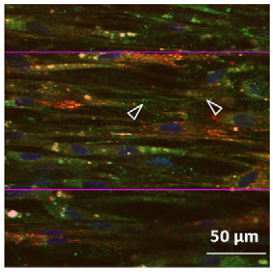

Fig. 1. Human MSCs were cultured for 21 days within collagen channels of 100 micrometers. Immuno-reactivity for collagen type II (green), 4′,6-diamidino-2-phenylindole (DAPI) counterstain of the nuclei (blue) Dil stain for cell membrane (red).

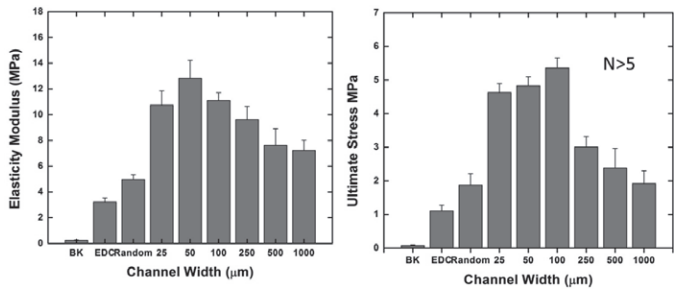

Fig. 2. Effect of microscale guidance on mechanical properties. hMSCs were cultured in collagen channels for 21 days in membranes containing channels of various dimensions (25–1000 μm). Unseeded (BK), unseeded crosslinked (EDC) and randomly seeded (Random) membranes were used as controls. Mean values of modulus of elasticity and ultramate stress are shown (n = 5/7 samples/condition).

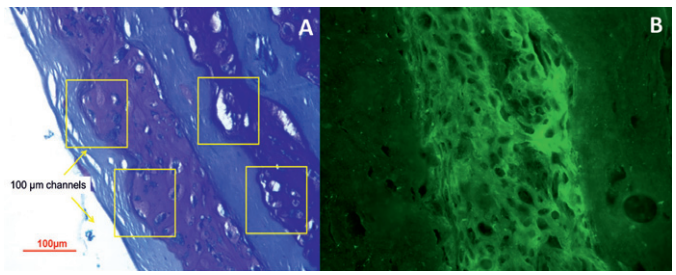

Fig. 3. Histology (toluidine blue staining, 3A) and immunohistochemistry (type II collagen staining, green, 3B) of constructs fabricated with microscale guidance channels.

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ISOLATION AND CHARACTERIZATION OF MULTI-POTENTIAL MESENCHYMAL CELLS FROM MOUSE SYNOVIUM

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Purpose: Human synovial mesenchymal stem cells are shown to have a higher capacity for proliferation and greater chondrogenic potential than those from other cell sources, such as bone marrow cells (BMCs) and muscle derived cells (MDCs). Thus, synovial mesenchymal stem cells are considered to be one of the appropriate candidates of cell sources for articular cartilage repair. However, numerous basic research questions related to the molecular mechanisms of tissue repair from these cells are also still largely unanswered. Mouse primary cell culture, in general, enables us to proceed the research for elucidating the molecular mechanisms of target phenomena because of a relatively easy for gene manipulation that is indispensable for the molecular analysis. However, one of the obstacles we are currently confronting is that mouse synovial mesenchymal cells (SMCs) are not available for basic research, whereas the rabbit, cow, and rat SMCs are available in addition to human mesenchymal stem cells.
The aim of this study was to establish methods to harvest synovium and to isolate and culture primary mesenchymal stem cells in mice.
Methods: Synovium was harvested from the infrapatellar fat pad of 10-week-old female Balb/c mice. The tissue was minced well to small pieces and given collagenase processing. Digested cells were filtered and cultured for 14 days. For flow cytometry, antibodies against CD29, CD34, CD45, CD117, and Sca-1 were used. As examination of the proliferation potential, growth kinetics and colony forming assay were performed. For *in vitro* chondrogenesis, 3×10^4 cells were placed in a polypropylene tube and centrifuged. The pellet was cultured in chondrogenic medium containing BMP7. For osteogenesis or adipogenesis, the cells were cultured in either calcification or adipogenic medium. For qRTPCR analysis the following primers were used: RUNX2 for osteogenesis, PPARγ for adipogenesis, Sox9 and Col2a1 for chondrogenesis. BMCs and MDCs were used as controls.
Results: As the mouse SMCs were not able to be harvested using the same protocol for human SMCs (Sekiya et al. Arthritis Rheum, 2006), the protocol for human needed the modification for mouse SMCs. The major modifications were the changes of collagenase treatment conditions, such as lesser concentration, shorter reaction time, and supplementation of DNase in collagenase solution. The proliferative potential of mouse SMCs was superior in comparison to that of both BMCs and MDCs. The colony-forming potential of mouse SMCs was similar to MDCs and superior compared to that of BMCs. The positive ratios of CD29, CD34, CD45, CD117, and Sca-1 in mouse SMCs were similar to those in both BMCs and MDCs. The positive ratios of CD44, CD106 and CD140a in mouse SMCs were higher than that in both BMCs and MDCs. The mouse SMCs were differentiated into cartilage *in vitro*. The pellets from mouse SMCs were larger and had amount of cartilage matrix shown by toluidine blue staining than those from both BMCs and MDCs. The osteogenic potential of mouse SMCs was similar to that of MDCs and superior to that of BMCs. The adipogenic potential of mouse SMCs was superior to that of both BMCs and MDCs.
Conclusions: These synovial mesenchymal cells harvested by the established method are expected to enable us to analyze the complex network of signaling pathways that very likely regulates their proliferative and differentiation potential of synovial mesenchymal stem cells by conducting *in vivo* analysis of genetically modified experimental models. In conclusion, we established primary mouse synovial cell culture method by determining the condition for isolation of the cells. The cells derived from mouse synovium demonstrated proliferation ability and multipotentiality, both characteristics of mesenchymal stem cells.

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EARLY INJECTIONS OF ADIPOSE-DERIVED STEM CELLS (ASCS) PROTECT AGAINST CARTILAGE DAMAGE AND LOWER SYNOVIAL ACTIVATION IN EXPERIMENTAL OSTEOARTHRITIS

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Purpose: Synovial activation is evident in a substantial subpopulation of patients with early osteoarthritis (OA) and has been associated with pathophysiology and clinical symptoms of OA. Previous studies have shown that synovial activation is involved in mediating cartilage destruction during experimental OA. Recently it has been shown that Adipose-derived Stroma/Stem Cells (ASCs) express immunosuppressive characteristics. The aim of our study was to explore the effect of intra-articular injection of ASCs on synovial activation and cartilage destruction during experimental OA.
Methods: ASCs were isolated from inguinal fat surrounding the popliteal lymph nodes and cultured for two weeks according to standard procedures. ASC were characterized with FACS analysis on a set of specific cell surface markers. Experimental OA was induced by injection of collagenase into murine knee joints, which causes instability and cartilage destruction. Collagenase-induced OA is characterized by thickening and activation of the synovial lining layer. ASCs were injected